6P21

Exploring the zinc binding site of mitochondrial complex I

Katarzyna Kmita, Ulrich Brandt, Klaus Zwicker, Volker Zickermann
Molecular Bioenergetics Group, Medical School, Cluster of Excellence Frankfurt "Macromolecular Complexes," Goethe-University, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany
E-mail: kmita@zbc.kgu.de

Respiratory complex I from the aerobic yeast *Yarrowia lipolytica* contains 14 central subunits intimately linked with energy conservation and at least 28 accessory subunits [1]. The accessory NUMM subunit is a member of the zf — CHCC superfamily (PF 10276; Pfam database) and carries a zinc finger motif: $CX_8HX_{14}CX_2C$ which is highly conserved in orthologous subunits found from alpha-proteobacteria to humans [2].

We have mutated all conserved residues of the putative zinc binding motif in the NUMM subunit from *Y. lipolytica* and investigated the impact on assembly and activity of complex I. Deletion of the subunit severely affected complex I assembly. Complementation with the subunit carrying a mutation of a cysteine previously reported to play a role in the pathogenesis of fatal neonatal lactic acidemia [3] rescued assembly but diminished ubiquinone reductase activity.

References

[1] H. Angerer, K. Zwicker, Z. Wumaier, L. Sokolova, H. Heide, M. Steger, S. Kaiser, E. Nübel, B. Brutschy, M. Radermacher, U. Brandt, V. Zickermann, Biochem. J. 437 (2) (2011) 279–288.
[2] C. Yip, M.E. Harbour, K. Jayawardena, I.M. Fearnley, L.A. Sazanov, J. Biol. Chem. 286 (2011) 5023–5033.
[3] R. Spiegel, A. Shaag, H. Mandel, D. Reich, M. Penyakov, Y. Hujeirat, A. Saada, O. Elpeleg, S.A. Shalev, Eur. J. Hum. Genet. 17 (2009) 1200–1203.

doi:10.1016/j.bbabio.2012.06.161

6P22

NADH-OH, a strong competitive inhibitor of complex I

Alexander B. Kotlyar[1], Gary Cecchini[2], Joel S. Karliner[2], Vera G. Grivennikova[3], Andrei D. Vinogradov[3]
[1]*Tel Aviv University, Ramat Aviv, 69978 Israel*
[2]*VA Medical Center and University of California San Francisco, CA 94121, USA*
[3]*Department of Biochemistry, School of Biology, Moscow State University, Moscow 119992, Russian Federation*
E-mail: s2shak@post.tau.ac.il

We discovered a very potent and specific inhibitor (NADH-OH) of mitochondrial complex I (NADH:ubiquinone reductase) [1]. The inhibitor is spontaneously formed during aerobic incubation of the reduced dinucleotide under alkaline conditions or in anaerobiosis in the presence of H_2O_2, and Fe^{+2} or Cu^{+2}. The latter suggests that the mechanism of NADH-OH formation includes modification of NADH with hydroxyl radical ($OH^{\cdot}$). The molecular mass of NADH-OH estimated by ESI-MS (696 Daltons) and preliminary 1H NMR data indicate that the inhibitor is derived from attachment of two oxygen atoms one to the adenine residue and the other to the nicotinamide residue of the inhibitor. NADH-OH is competitive with respect to NADH with a Ki of about 10^{-8} M. The inhibitor efficiently inhibits NADH-oxidase, NADH-artificial acceptor reductase, and NADH-quinone reductase reactions catalyzed by submitochondrial particles, as well as the reactions catalyzed by either isolated complex I or the three subunit flavoprotein fragment of complex. The inhibitor also strongly suppressed a succinate-supported superoxide generation by complex I [2].

The structural similarity of NADH-OH and NADH, the high potency and specificity of the inhibitor with respect to complex I and its ability to suppress ROS formation [2] raise the exciting possibility that it is a natural derivative of NADH that can modulate activity of complex I in normal conditions and under periods of oxidative stress. It may be possible that NADH-OH could be produced in mitochondria by enzymatic hydroxylation of the nicotinamide ring of NADH under certain metabolic conditions. If indeed the inhibitor can be produced in cells this would provide important insights for understanding the mechanism of complex I regulation in vivo.

[1] Kotlyar A., Karliner J., Cecchini G., (2005) *FEBS Letters* **579**: 4861-4866.
[2] Grivennikova V.G, Alexander B. Kotlyar A. B., Joel S. Karliner J. S., Cecchini G., Vinogradov A. D. (2007) *Biochemistry* **46,** 10971-10978.

doi:10.1016/j.bbabio.2012.06.162

6P23

Complex I studied by Surface Enhanced IR Absorption Spectroscopy (SEIRAS) and electrochemistry

Sébastien Kriegel[1], Frédéric Melin[1], Thorsten Friedrich[2], Masatoshi Osawa[3], Petra Hellwig[1]
[1]*Institut de Chimie UMR 7177, Laboratoire de spectroscopie vib. et electrochimie des biomolecules, CNRS-Université de Strasbourg, 1, rue Blaise Pascal, 67070 Strasbourg, France*
[2]*Institut für Organische Chemie und Biochemie, Albert-Ludwigs-Universität Freiburg, Albertstr. 21, 79104 Freiburg, Germany*
[3]*Catalysis Research Center and Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 06-0811, Japan*
E-mail: skriegel@unistra.fr

The work presented deals with the use of Fourier Transform Infrared spectroscopy (FTIR) applied to biomolecules, especially to the first enzyme of the respiratory chain, the NADH:ubquinone oxidoreductase, also called Complex I. Complex I couples the oxidation of NADH into NAD^+ to the reduction of ubiquinone into ubiquinol. The electrons gained from the oxidation of NADH are transferred to the quinone through one FMN and up to 9 Fe/S clusters. The process is coupled to the translocation of 4 protons across the membrane by a mechanism involving long-range conformational movements. This process is not yet fully understood, although recent X-ray structure [1] revealed that it seems to function in a piston-like manner.

Complex I is a primary target for herbicides and is thought to be involved in various diseases, e.g. Parkinson disease or Alzheimer's disease, and in aging [2]. To study its role in these processes and to gain new insights in the structure–function relationship of the protein, a method to create a molecular probe is proposed here. It involves FT-IR absorption spectroscopy in combination with other techniques, as electrochemistry and perfusion [3]. Particular attention is given to Surface Enhanced IR Absorption Spectroscopy (SEIRAS) [4], a variation of Attenuated Total Reflection (ATR) IR Spectroscopy. SEIRAS uses a thin gold layer deposited on top of a Si crystal to enhance IR absorption. This gold layer provides a convenient way to attach molecules on its surface and can also be used as a working electrode for electrochemical experiments.

References

[1] R.G. Efremov, L.A. Sazanov, Nature 476 (2011) 414–422.
[2] F. Distelmaier, et al., Brain 132 (2009) 833–842.